DIFFERENTIAL ABSORPTION OF LEAD IN 20 SOILS FROM SUPERFUND MINE-WASTE SITES:

Measures of Bioavailability Using
Juvenile Swine as a Model of Young Children
(with physiologically based variations in responses)

Conference on Topics in Toxicology and Risk Assessment
Air Force Research Laboratory, WPAFB
April 15, 1999

G Henningsen, DVM PhD[1]; C Weis, PhD[1]; S Casteel, DVM PhD[2]; L Brown, DVM PhD[2]; E Hoffman, PhD[1]; W Brattin, PhD[3]; J Drexler, PhD[4]; S Christensen[1]

[1]USEPA Region VIII, Denver, CO; [2]University of Missouri, Columbia, MO;
[3]R.F. Weston, Inc., Lakewood, CO; and the [4]University of Colorado, Boulder, CO.

Why Measure Bioavailability of Lead?

- Site-specific adjustments of default absorption: improve dose estimates for risk assessments
- Large-area Superfund mining sites: incentives to minimize high costs and public disruptions
- Large uncertainties in existing data-base: ages, rodents, high doses, sieved soil, solubility tests
- EPA guidances: use best available science

Experimental Designs for Bioavailability

- *Representative* of residential child's exposure to soil-lead
  - particle sizes <250 um (sieved at 60 mesh) adhere to hands
  - yard locations representing most typical contact with soil-lead
  - geophysicochemistry of metallic salts in an aged soil matrix

- Solubility tests alone cannot predict bioavailability
  - **bioavailability** / degree that a chemical is absorbed and available to the target tissue after administration; i.e., lead's effects on developing nervous tissues, blood is a biomarker

    "**absolute**" (total uptake) vs "**relative**" (fraction of reference chemical)
  - **bioaccessibility** / amount of chemical that contacts a biological surface (membrane) in a form that has potential to be absorbed

EPA R8's Approach and Study Objectives

Phase I:

Fully characterize the immature swine model

dose- and time-dependence of juvenile absorption of Pb Acetate (soluble salt as reference chemical)

Phase II:

Quantify the absorption characteristics of lead

wide spectrum, fully characterized mixtures of soil

- from EPA Superfund sites with mine wastes
- residential exposures to young children

Associated Pilot Studies on Pb Soils

(to strengthen interpretation of results)

- Kinetics of acute and subchronic clearance
- Absorption during fed and fasted states
- GI transit time in the immature swine model
- Maternal to fetal transfer
- Inter-species comparisons of absorption
- Mechanisms of lead absorption: is GI uptake saturable (non-linear) or non-saturable (linear)?


522

General Subchronic Study Design

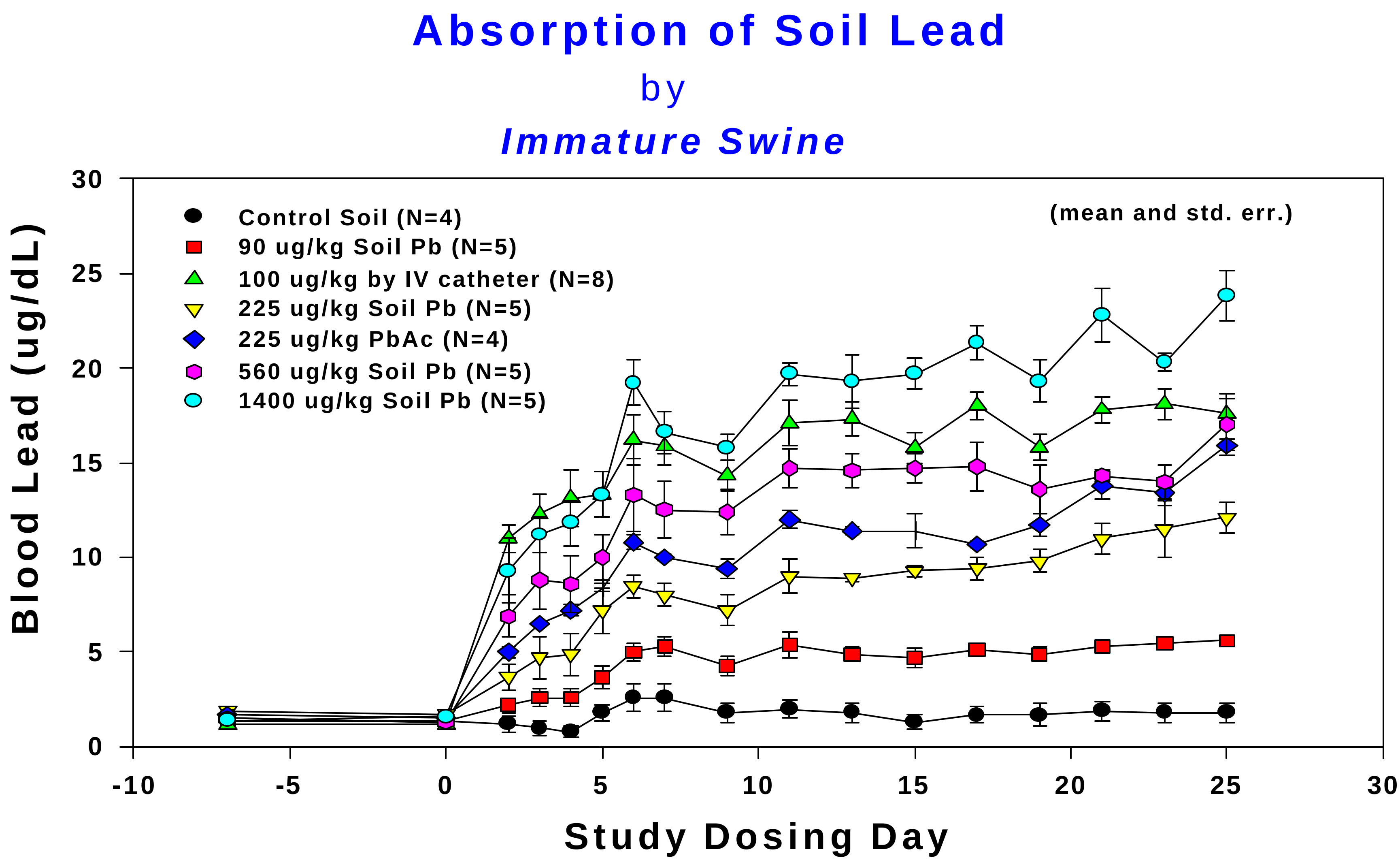
Group	N	Treatment	Acetate/Soil Lead	Target Dose (ug/kg-d)
1	2	Vehicle Control	Vehicle Only	0
2	5	Pb(AC)	Weight Adjusted	75
3	5	Pb(AC)	Weight Adjusted	225
4	5	Soil 1	Mass and weight adjusted	75
5	5	Soil 1	Mass and weight adjusted	225
6	5	Soil 1	Mass and weight adjusted	675
7	5	Soil 2	Mass and weight adjusted	75
8	5	Soil 2	Mass and weight adjusted	225
9	5	Soil 2	Mass and weight adjusted	675
10	8	IV Pb(Ac)	Weight adjusted	100

QA/QC: EPA Q.A.P.P. (protocol and S.O.P.s), Chain of Custody; GLP; double-blind random samples; P.A.R.C.C. criteria; and Peer-Reviewed

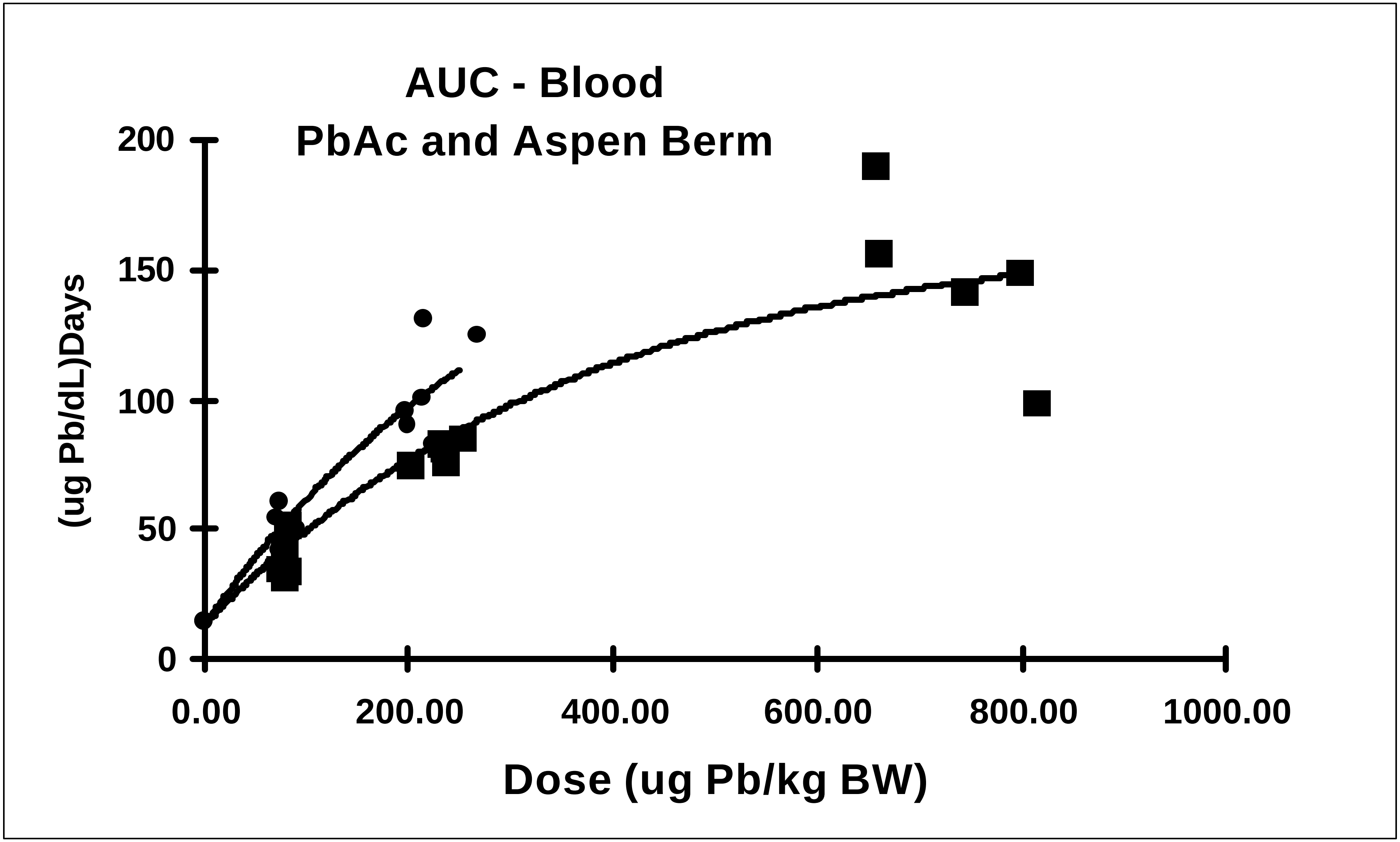
Dosing Regimen for Steady-State Blood-Pb

- Animals were dosed 2 hours before each feeding, twice (split doses) daily, 15 days, constant times (attained quasi-steady-state blood-lead levels)
- Oral doses of soil-lead or lead-acetate were given in a small 5 gram “dough ball” of moistened low-lead feed
- IV lead-acetate was administered by a sub-cutaneous sterile “vet port”, surgically implanted into vena cava.

Phase I Characterization of Dose and Time Responses for Absorption of Lead Acetate and a Test Soil-Lead

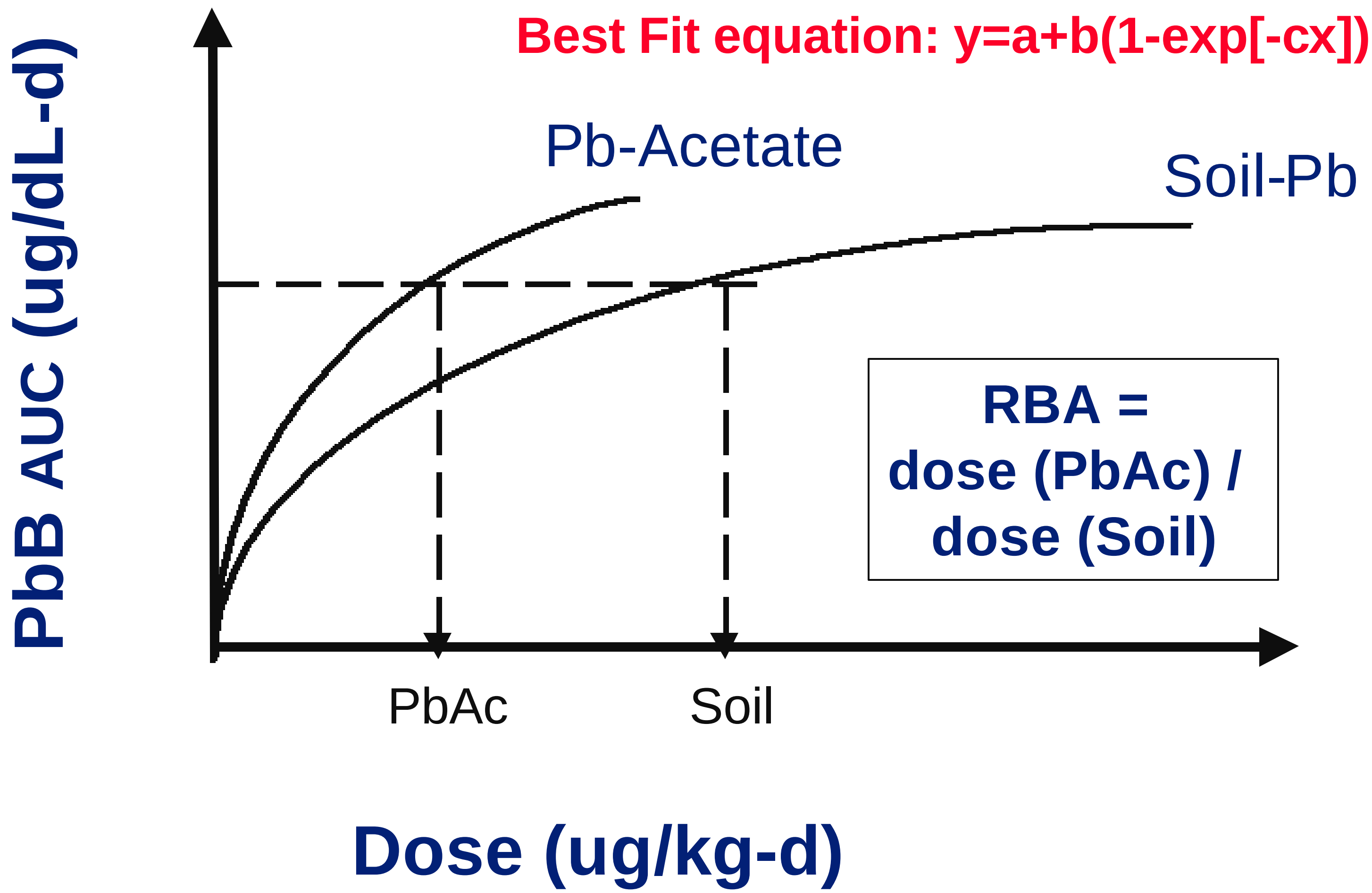


EXAMPLE: Site-Specific RBA Estimation



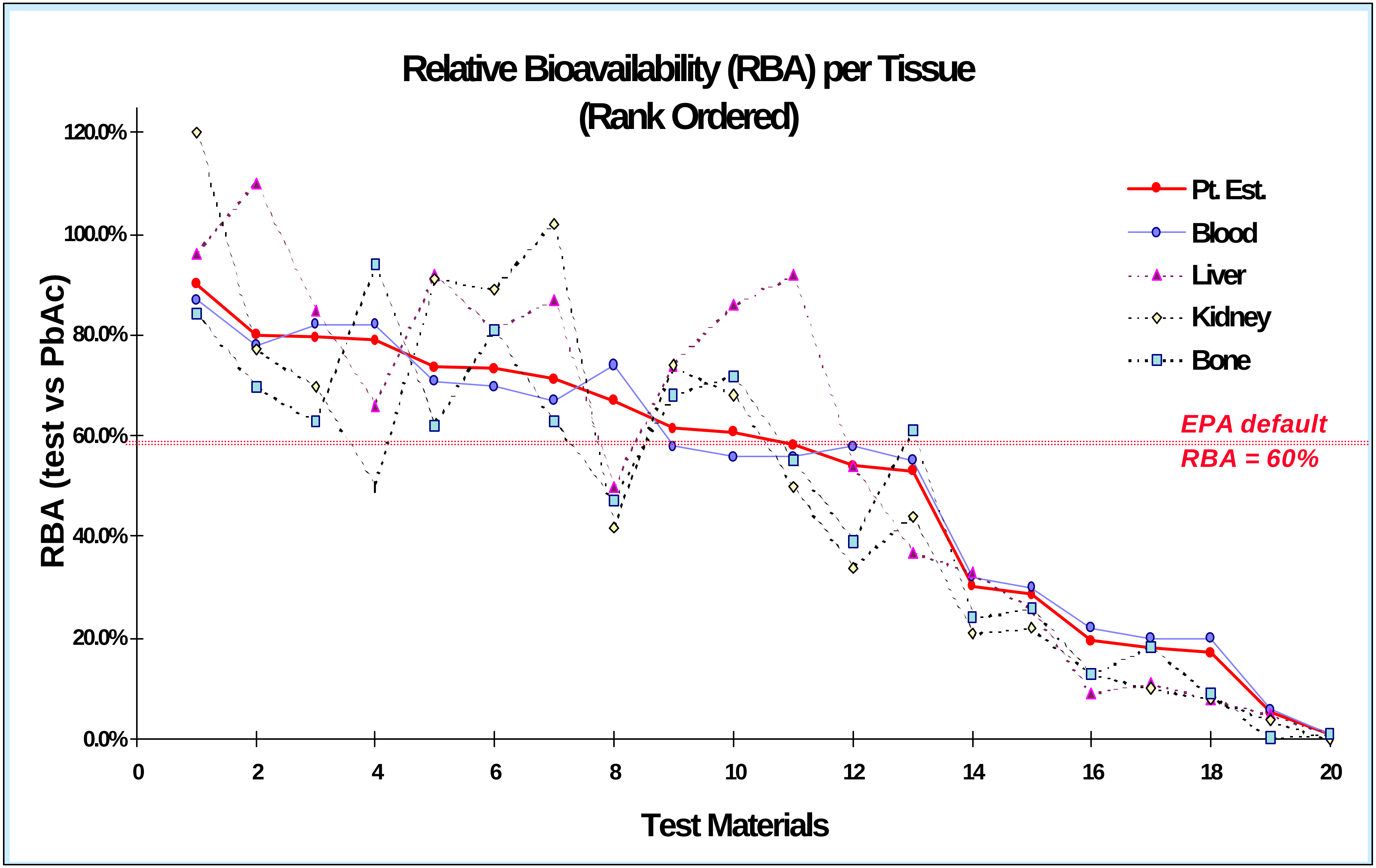
CALCULATION: *Relative* Bioavailability

RBA *= "Ratio of doses" for any single* ***non-linear*** *response*



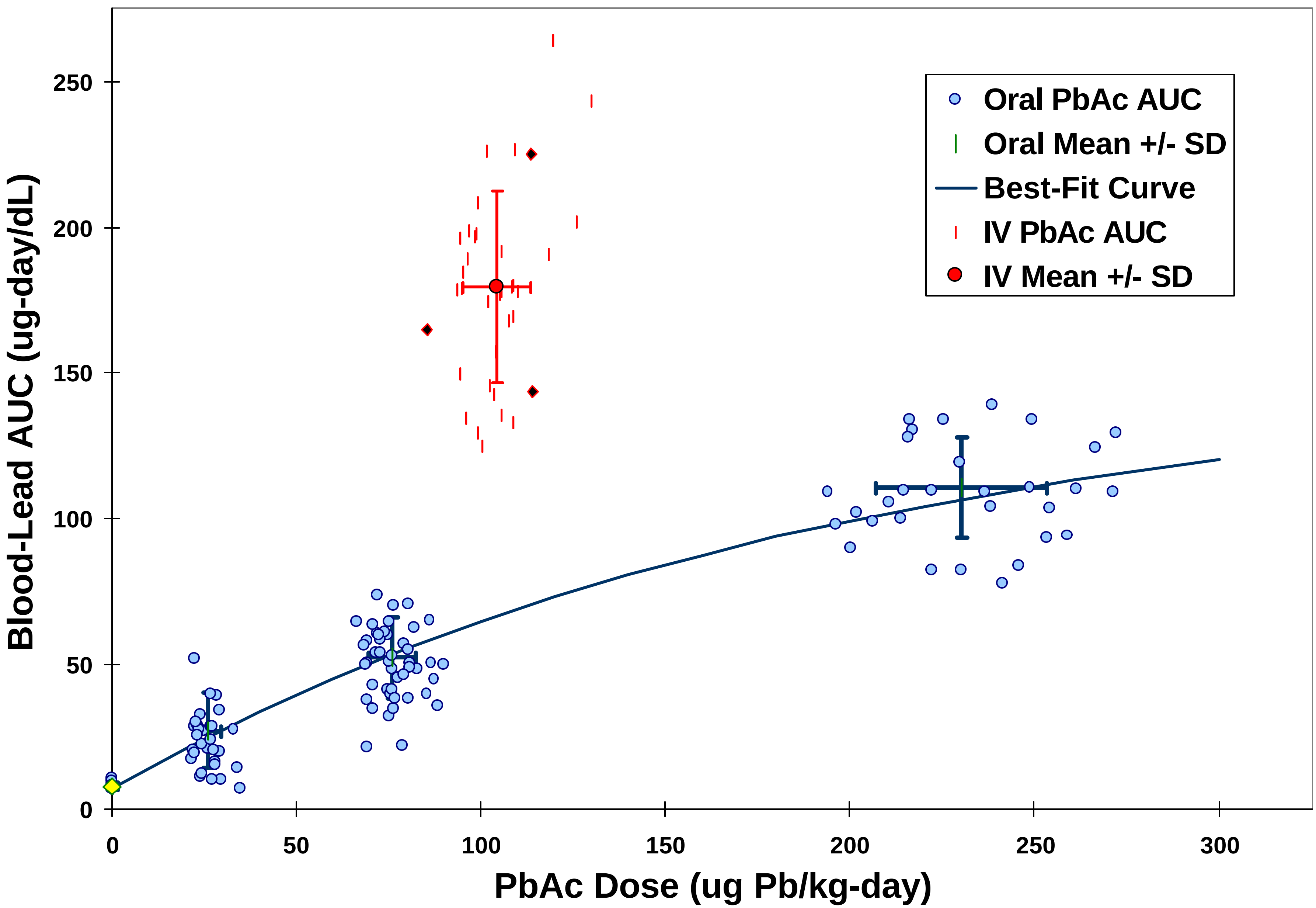
RBA = dose (PbAc) / dose (Soil)

RBA ESTIMATES: Soil-Lead at 20 Sites

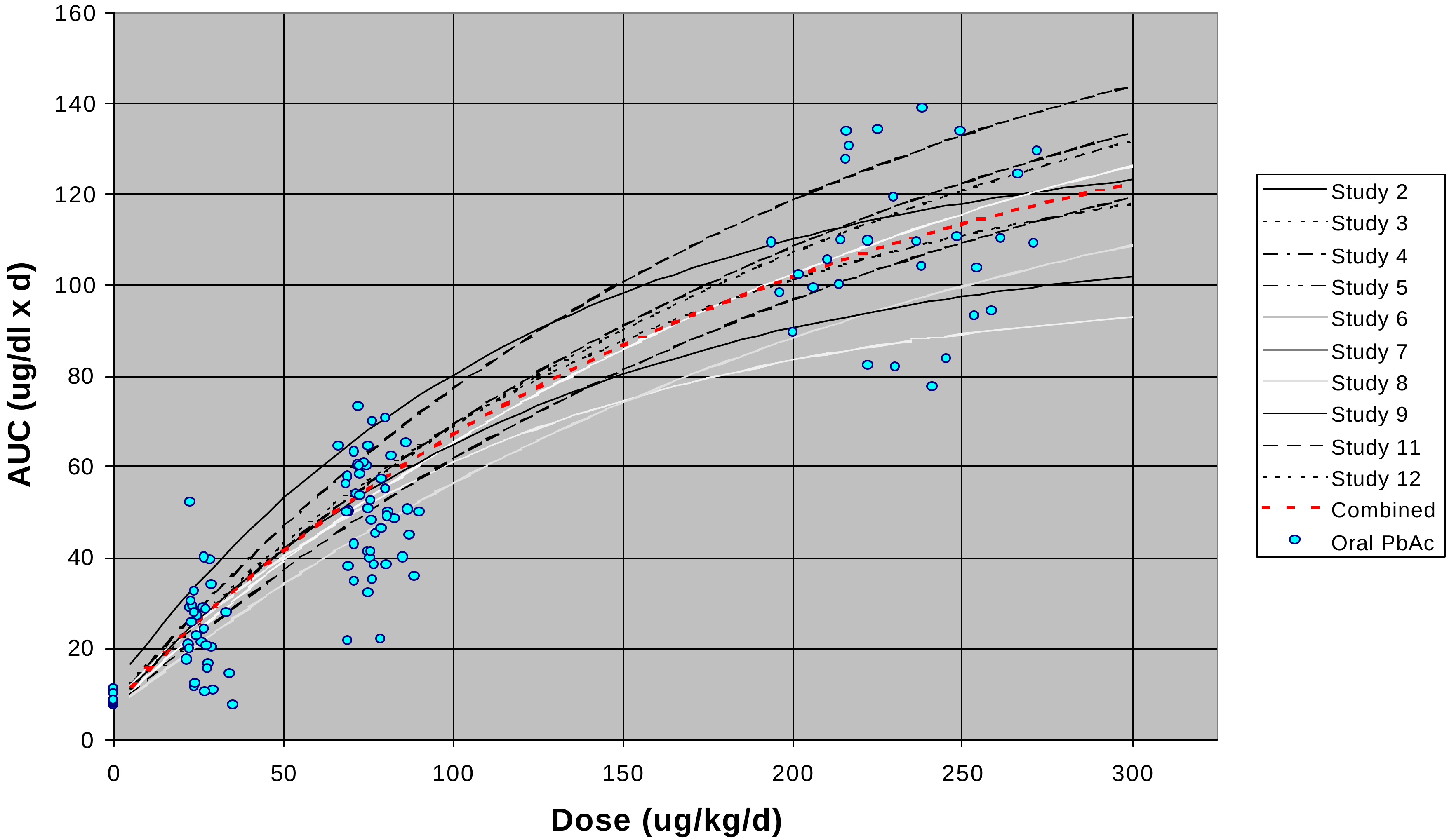


PbAc Dose-Response Over 10 Studies

Blood-Lead AUC of Individual Control Animals



PbAc Control Curves for EPA Pig Studies



SUMMARY OF RESULTS

Bioavailability Studies with Lead in Young Swine Model

- The juvenile swine model is **reasonably stable** over studies, but enough positive (PbAc) and negative (vehicle) internal-<u>controls are necessary</u> to obtain confident site-specific RBAs due to the inherent variability observed among weanling pigs

- **Accuracy and precision** of analyses for lead in tissues were good: generally <u>+/- 5-10% RPD, and the MDL = 1 ug/dl</u>; pigs can *sustain repeated blood sampling* of 3-10 ml per draw

- The **adjustment of doses** every 3 days for increasing weights of growing pigs (0.5kg/d) permits accurate and stable dosages

- Studies repeated on two soil-lead: RBAs were **reproducible**: 73 vs 75% within Phase II, 77% vs 74% for Phase II vs I (inter-laboratory)

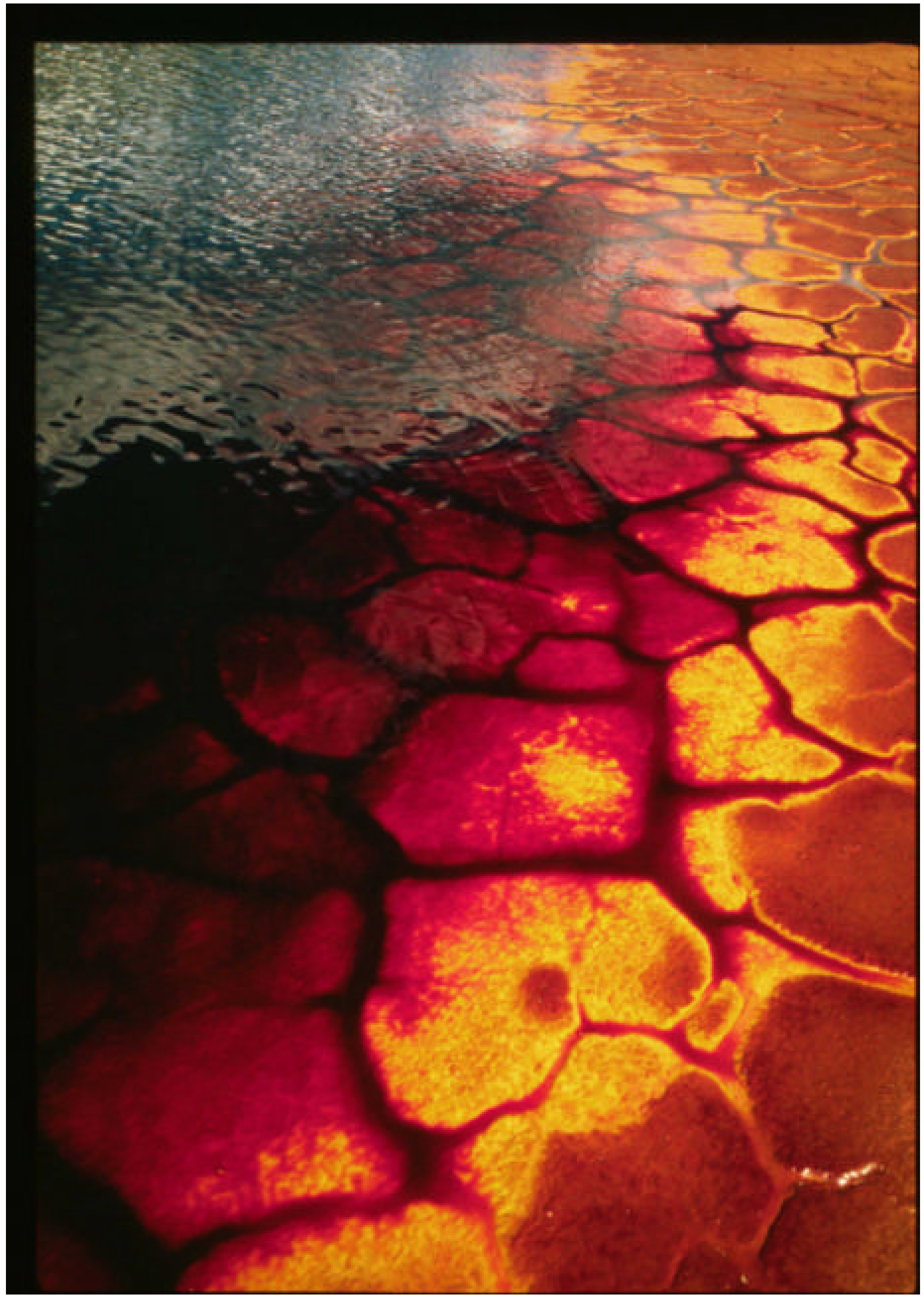
- **Geophysical-chemical characterization** by electron microprobe fluorescence provided useful data on soil-lead particles for their frequency, size, mass, Pb-ion speciation, and mineral matrix

- **Results** for 20 soil-leads, in respect to EPA's 60% default RBA, are:

  higher RBAs (>75%) are associated with $PbCO_3$ and PbMn(M)O

  average RBAs (25% - 75%) are associated with PbO, PbFe(M)O, $PbPO_4$, and Pb-Slags

  lower RBAs (<25%) are associated with PbS, $PbSO_4$, Pb(M)O, $PbFe(M)SO_4$, and metallic Pb



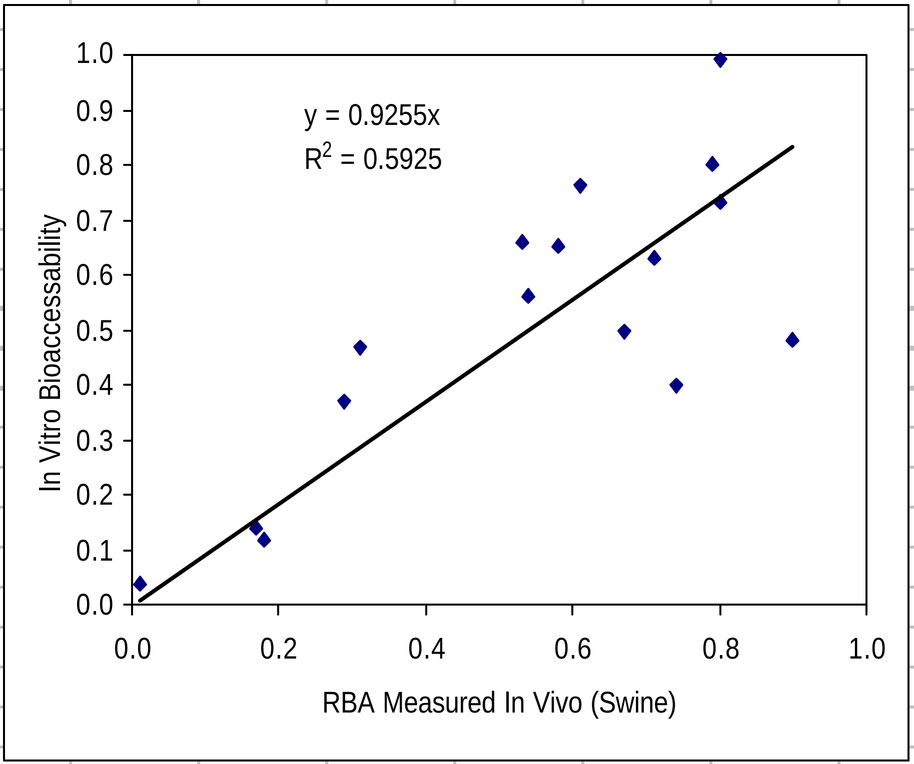
CONCLUSIONS

- The immature **swine model is a useful and valid tool** that estimates the oral absorption of soil-lead mixtures
- **20 Superfund soils had RBAs ranged from 86% to 3%** (feeding pigs during dosing reduced absorption by about half)
- EPA's **default RBA = 60% may over- or under-estimate** the actual absorption of oral exposures to soil-lead
- Results are useful for helping to develop validated in-vitro tests of bioaccessibility that correlate with bioavailability

In Vitro Correlation of PbB

Preliminary, Draft, Un-validated Results

Material	CLP Pb Analysis	In Vivo	CU In Vitro
Aspen Berm	14,200		
Aspen Residential	3870	0.61	0.76
Palmerton Location 2	3230	0.67	0.50
Palmerton Location 4	2150	0.54	0.56
Jasper HL Smelter	10,800	0.58	0.65
Jasper LL Yard	4050	0.80	0.73
Jasper HL Mill	6940	0.79	0.80
Murray Smelter Slag	11,700	0.53	0.66
Murray Smelter Soil	3200	0.71	0.63
Butte	8585		
Midvale Slag	7895	0.17	0.14
Bingham Creek LL Soil	1590	0.31	0.47
Bingham Creek HL Soil	6330	0.29	0.37
NIST Paint	8350	0.80	0.99
Galena	11,200	0.01	0.04
Leadville Residential Comp.	7510	0.74	0.40
Leadville Fe-Mn-Pb Oxide	4320	0.90	0.48
AV Slag	10,600	0.18	0.12
Oregon Gulch Tailings	1270		

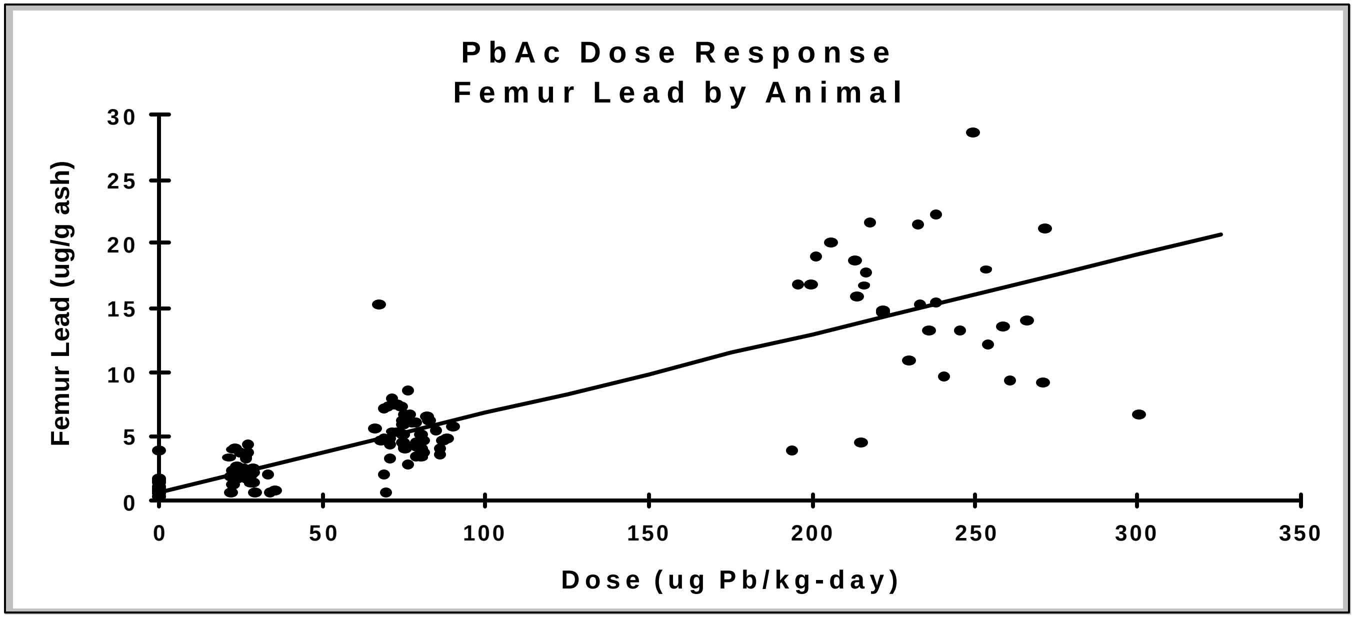


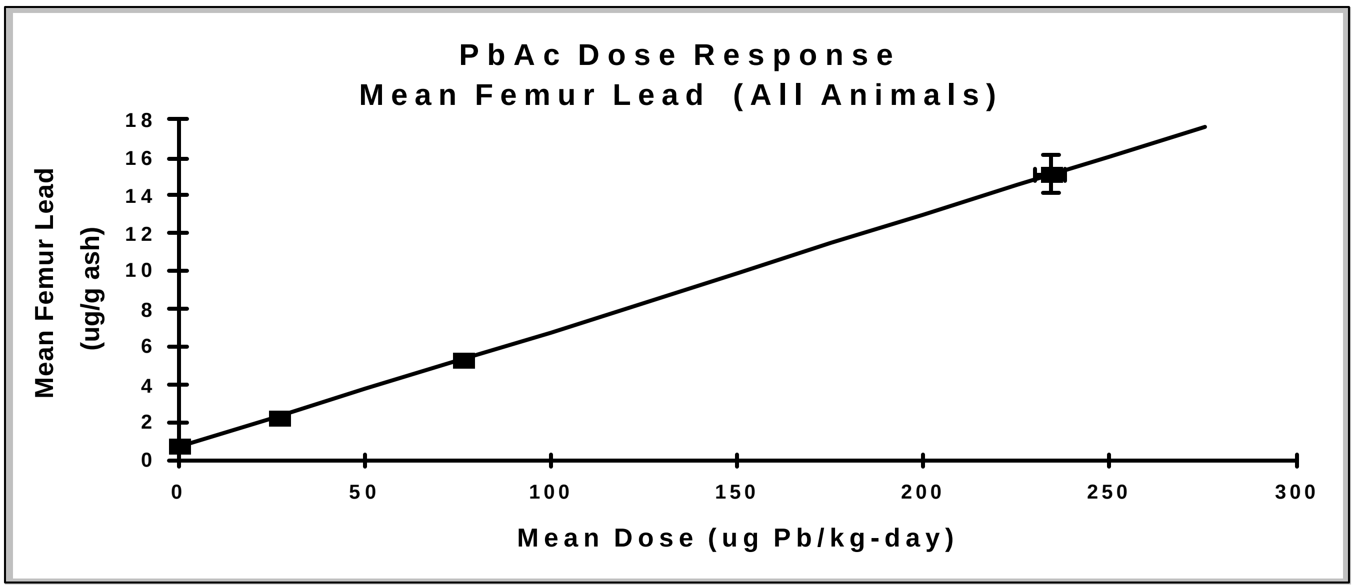
Physiological Aspects of Absorption of Pb in Pigs

Q: Is GI-Uptake of Pb into Blood *Saturable or Linear*?

- The response to lead-doses is **non-linear** (saturable) **in blood** following both oral **and IV** dosing over 15 days
- Responses to lead-doses in other tissues **(bone, liver, kidney) are linear** for both oral and IV over 15 days
- Conclude: ***non-linearity*** *of kinetic uptake of lead occurs in mammalian* ***blood*** *compartment; NOT at the level of gastro-intestinal* ***absorption***

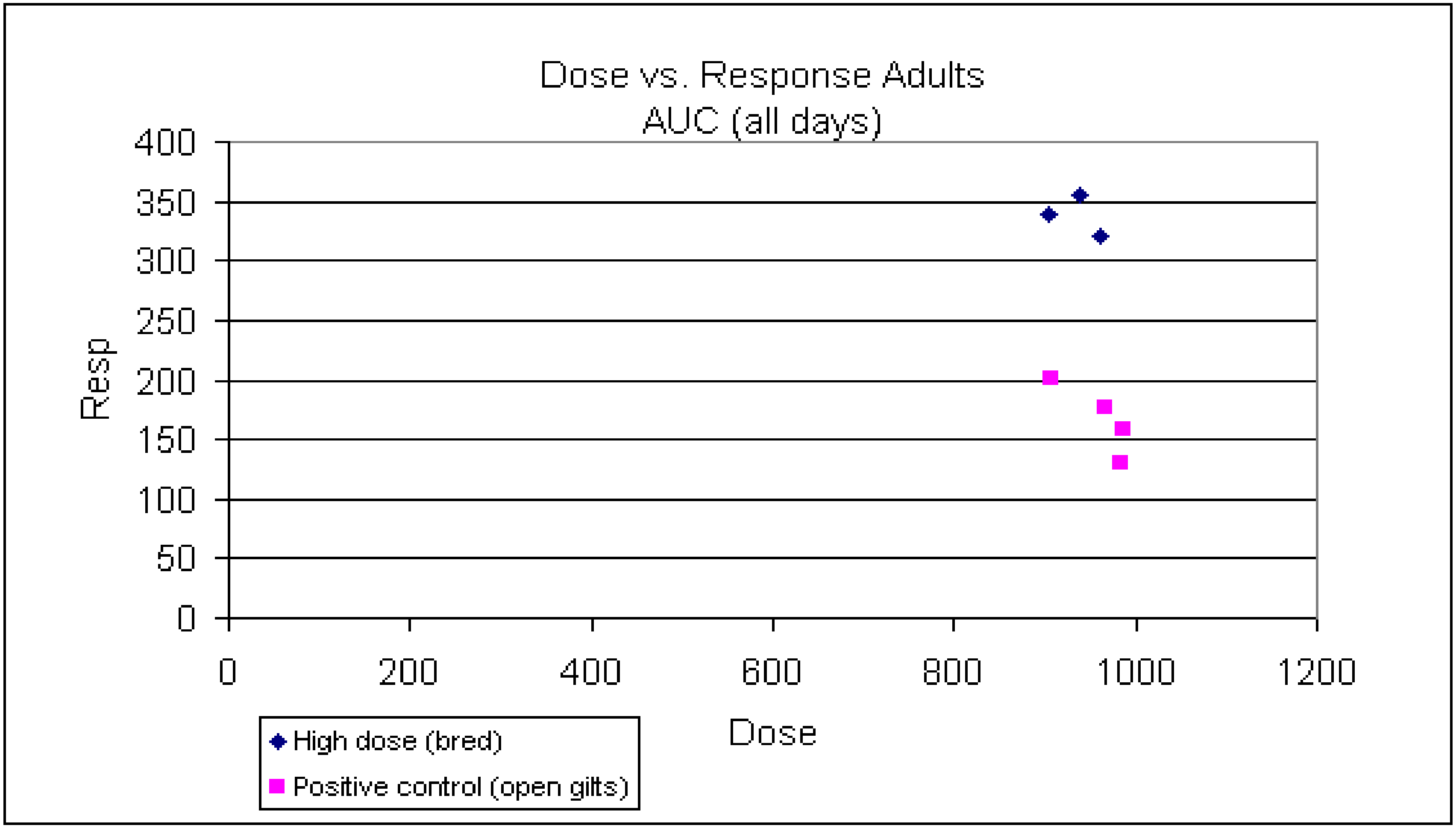
BONE RBA: All Pigs Dosed with PbAc

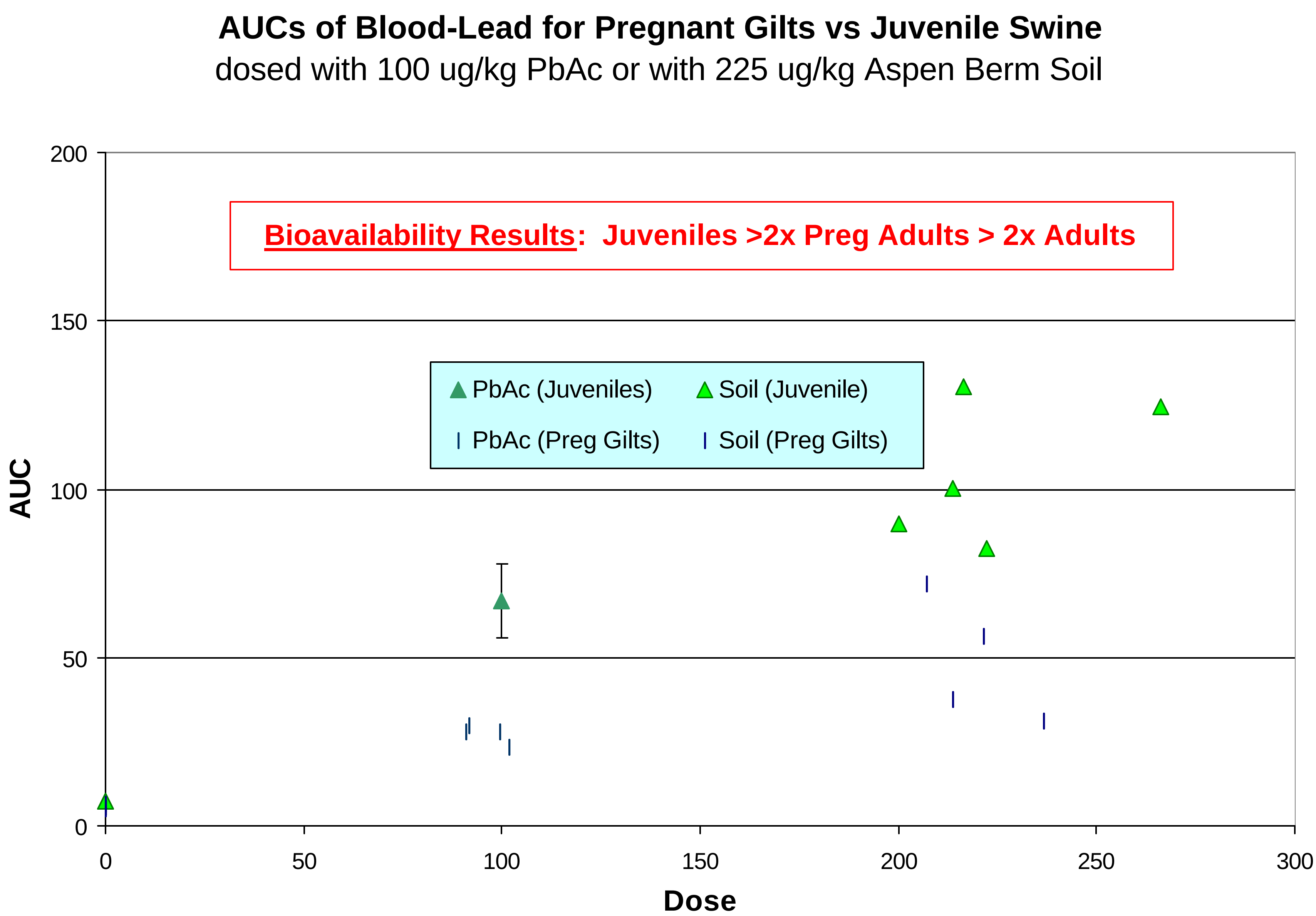




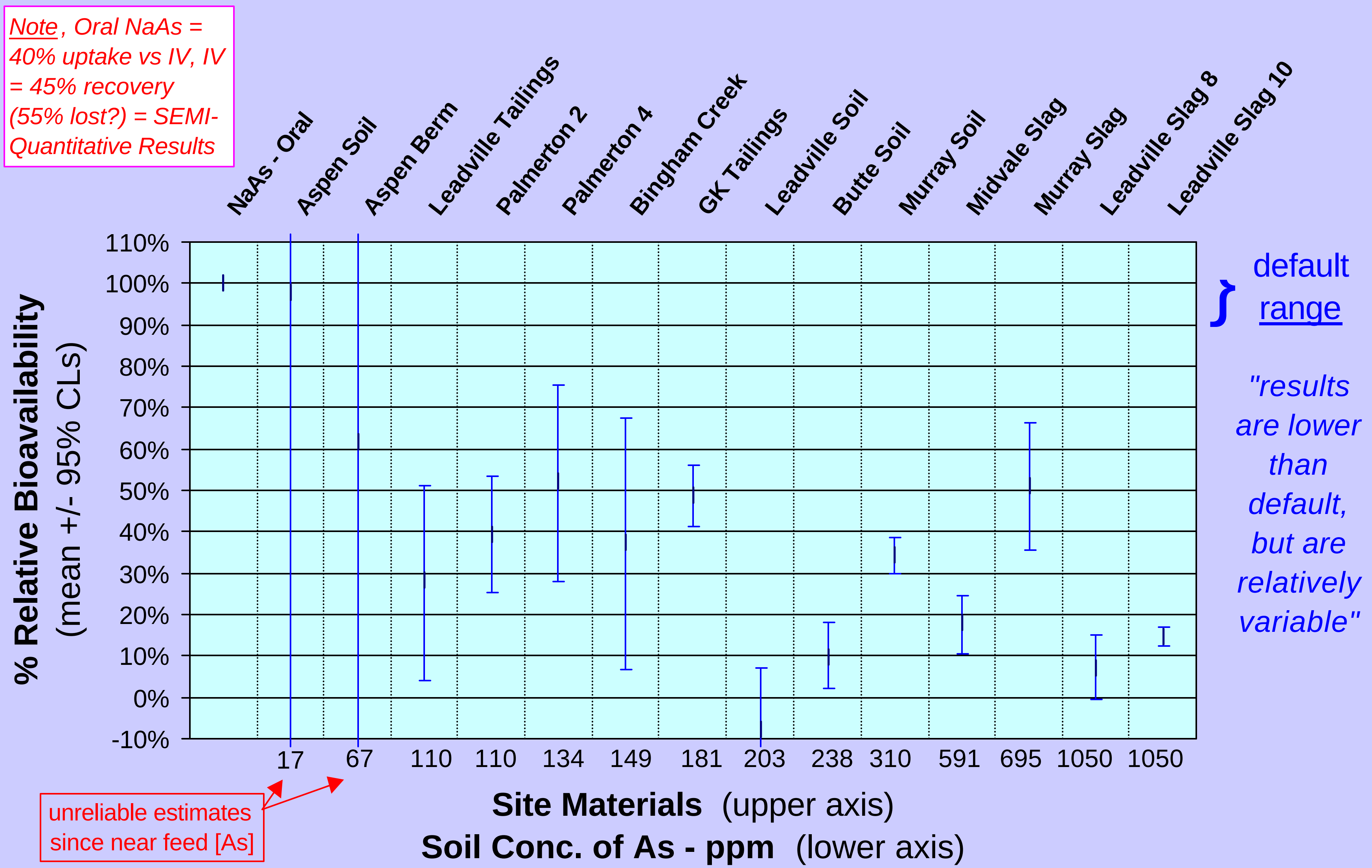
Greater Uptake During Pregnancy

Fetus PbB about 90% of Maternal – from Diet




AUCs of Blood-Lead for Pregnant Gilts vs Juvenile Swine
dosed with 100 ug/kg PbAc or with 225 ug/kg Aspen Berm Soil
Bioavailability Results: Juveniles >2x Preg Adults > 2x Adults
PbAc (Juveniles)
Soil (Juvenile)
PbAc (Preg Gilts)
Soil (Preg Gilts)
AUC
Dose
0
50
100
150
200
250
300

RBA in Swine (urine) of Arsenic in Mine Wastes



Ecological Risk Assessment Needs for Bioavailability Data

Like for human health, bioavailability studies can evaluate wildlife **assimilation** of contaminants from abiotic and biotic media:

frequently measured as “relative effects”, including absorbed dose

Focus is often on terrestrial wildlife and **bioaccumulation** from food-web ingestion pathways, vs more straight-forward aquatic **bioconcentration** or bioconcentration factors (BCFs) for plants

Needs go beyond simple exposure assessments of “contact”, towards how much contaminant becomes **absorbed** by wildlife

Site conceptual models are often more *complex* than for humans, since multi-media exposure pathways include food chains & webs